

JPred4: a protein secondary structure prediction server

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ABSTRACT

JPred4 (<http://www.compbio.dundee.ac.uk/jpred4>) is the latest version of the popular JPred protein secondary structure prediction server which provides predictions by the JNet algorithm, one of the most accurate methods for secondary structure prediction. In addition to protein secondary structure, JPred also makes predictions of solvent accessibility and coiled-coil regions. The JPred service runs up to 94 000 jobs per month and has carried out over 1.5 million predictions in total for users in 179 countries. The JPred4 web server has been re-implemented in the Bootstrap framework and JavaScript to improve its design, usability and accessibility from mobile devices. JPred4 features higher accuracy, with a blind three-state (α -helix, β -strand and coil) secondary structure prediction accuracy of 82.0% while solvent accessibility prediction accuracy has been raised to 90% for residues <5% accessible. Reporting of results is enhanced both on the website and through the optional email summaries and batch submission results. Predictions are now presented in SVG format with options to view full multiple sequence alignments with and without gaps and insertions. Finally, the help-pages have been updated and tool-tips added as well as step-by-step tutorials.

INTRODUCTION

Knowledge of a protein's three-dimensional structure is central to understanding the protein's detailed function. Although recent developments in structural biology (1–4) have led to an acceleration in the rate of three-dimensional structure determination by X-ray crystallography, nuclear magnetic resonance and 3D-EM techniques, in January 2015 there were still just 105 732 protein structures known (<http://www.ebi.ac.uk/pdbe>) (5) compared to almost 90 million sequences (<http://www.ebi.ac.uk/uniprot/TrEMBLstats>) (6). The routine use of massively parallel DNA sequencing technologies today means knowledge of protein sequences will continue to outpace structural biology for the foreseeable

future. As a consequence, there is a need for accurate methods to predict structural and functional features from the amino acid sequence. Over the last 30 years, techniques to predict the three-state secondary structure of the protein (α -helix, β -strand and coil: i.e. all other states) have increased in accuracy from around 50% in 1983 (7) to over 80% today (8–11) which is close to the estimated maximum for prediction from multiple alignment (12). Although knowledge of the secondary structure alone is not as useful as a full three-dimensional model, secondary structure predictions provide important constraints for fold-recognition techniques (13–17) as well as in homology modelling (18,19), *ab initio* (20–24) and constraint-based tertiary structure prediction methods (25–27). Secondary structure predictions can also help in the identification of functional domains and may be used to guide the rational design of site-specific or deletion mutation experiments.

Although hundreds of papers have been published describing methods for protein secondary structure prediction, three of the most widely used are JPred, PSIPRED and PredictProtein. JPred (v. 3.0) (11) gave 81.5% three-state accuracy (Q_3), PSIPRED v.3.0 (28) reported accuracy of 81.4%, while the current PSIPRED V 3.2 server, which includes a broad suite of prediction algorithms, quotes 81.6%. (<http://bioinf.cs.ucl.ac.uk/psipred>). There is no recent blind prediction test for the PROFphd secondary structure prediction algorithm in the PredictProtein (29) secondary structure prediction method, though the earlier PROFsec reported 76% (30).

In this paper we summarize the current performance and features of the upgraded JPred server (JPred4) which incorporates the secondary structure and solvent accessibility prediction program JNet v.2.3.1.

MATERIALS AND METHODS

The basic usage pattern for JPred4 is the same as for JPred3 (11). The user can submit a single protein sequence, a multiple sequence alignment (MSA) or a batch of single protein sequences for prediction. Results are returned either interactively through a web page or as a summary email that directs the user to results on the JPred4 website.

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The screenshot shows the JPred4 web server interface. At the top, the title "Jpred 4 Incorporating Jnet" is displayed. Below it, the subtitle "A Protein Secondary Structure Prediction Server" is shown. A navigation bar with links to Home, About, News, F.A.Q., Help & Tutorials, Contact, and Publications is present. The main form area has the following fields:

- Input sequence**: A text input field containing a protein sequence: MQVWPPIEGIKKFETLSYLPPPLTVEDELLKQIEYLLRSKWVPCLEFSKVGFYRE...EYYDGRYWT. A tooltip (circled 3) explains the required input format: "JPred requires input as either a single sequence in Raw or FASTA formats ([link to format examples](#)), and pasted into the text box. All other inputs/options including a multiple sequence alignment or batch submission could be used through advanced options. [Show/hide more...](#)".
- Advanced options (click to show)**: A link that, when clicked, reveals additional options like file upload (circled 4).
- Select type of input**: A dropdown menu with two options: "Single Sequence (click to select format)" (radio button selected) and "Multiple Alignment (click to select format)".
- Skip searching PDB before prediction**: A checkbox labeled "Check to skip".
- Email address (optional)**: An input field containing "name@domain.com".
- Query name (optional)**: An input field containing "testJob".
- Buttons**: "Make Prediction" and "Reset Form".

To the right of the main form, there is a sidebar with the text "4 g Jnet Prediction Server" and links to "Contact" and "Publications". Below the main form, a "Job Status" section displays:

- "Your job (jp_OhwndFY) started at 16:41 on 23/01/2015, the current time is 16:43 on 23/01/2015."
- A progress bar indicating "10% complete..." (circled 7). A tooltip for the progress bar says "See below for a more detailed log of progress (use your back button to return): [Log file](#)".
- A message: "If the job is taking too long it may be worth deleting and resubmitting it using an e-mail address: [delete job](#)" (circled 8).

Figure 1. (1) Screenshot of the JPred4 job submission page with single sequence submission field (2) and an example of a tool-tip message (3). Advanced options are opened on request (4) and include input file upload, format selection (5) as well as optional email and query name fields (6). (7) Job progress page with access to the detailed job run log file (8).

The look and feel of the JPred4 web server has been changed significantly compared to JPred3 by embracing contemporary web technologies, the Bootstrap framework (www.getbootstrap.com/) and custom JavaScript. These changes allow smoother user interaction through the use of ‘ tooltips’ that pop up to present help on each option in an easy-to-read form without the need to leave the page. The Bootstrap framework provides a modern look and feel to the website as well as improving usability on devices such as tablets and phones with different screen sizes and resolutions. Figure 1 illustrates the appearance of the advanced submission page showing the use of tooltips to get help about each option. As well as updates to the help pages,

step-by-step tutorials with screenshots are a new addition that helps users to obtain maximum benefit from the JPred4 server.

Prediction algorithm

As with JPred3, JPred4 makes secondary structure and residue solvent accessibility predictions by the JNet algorithm (11,31). However, in JPred4, the JNet 2.0 neural network-based predictor has been retrained to make JNet 2.3.1 by 7-fold cross-validation using one representative for each of the 1358 SCOPe/ASTRAL v.2.04 superfamily domain sequences (32). Multiple alignments for each sequence were built by PSI-BLAST (33) through searching UniRef90

Jpre Incorpor.

A Protein Secondary Structure Prediction Server

Home About News F.A.Q. Help

Results

After much trouble and strife, Bob the scheduling your pleasure the following viewing options are available for reference although data is not kept on the server for

- View results summary in SVG - displayed below:

Notes
Key:
Colour code for alignment:
Blue - Complete identity at a position
Shades of red - The more red a position is, the higher the level of conservation of chemical properties of the amino acids
Jnet - Final secondary structure prediction for query
jalign - Jnet alignment prediction
jhmm - Jnet hmm profile prediction
jpssm - Jnet PSIBLAST pssm profile prediction
Lupas - Lupas Coil prediction (window size of 14, 21 and 28)

Note on coiled coil predictions - = less than 50% probability
c = between 50% and 90% probability
C = greater than 90% probability

- View full results in HTML
- View simple results in HTML
- View results in PS
- View results in PDF
- View results in Jalview (Links to a separate page with gaps)
- View full multiple sequence alignment with gaps
- View full multiple sequence alignment without gaps
- View everything in a results directory
- Get all (but PS) files in TAR.GZ archive

Figure 2. JPred4 results summary page (1) with the results of predictions presented in SVG (2). Links to detailed and simple reports in coloured HTML/PS/PDF formats (3). Example summary in HTML format is shown in (4) as well as the new addition of full multiple sequence alignments with and without gaps/insertions (5). On a separate linked page the user is able to run the Jalview applet (6) which allows a more sophisticated and interactive method of viewing the prediction results. Links to all the details for the prediction and an archive of the results are also available (7).

Jpred job jp_yrbsHd9 completed

 www-jpred@compbio.dundee.ac.uk
to me

Your job has completed successfully and the prediction is summarised below:

Query: MQVWPIEGIKKFETLSYLPLTVEDLLKQIEYLLRSKWVPCLEFSKVGTVRENHRSPGY
Jpred: ——EEE—HHHHHHHHHHHH—EEE—
Conf: 8853556775232117777627899999998745872667743677765456677777

Query: YDGRYWWTMWKLPMFGCTDATQVLKELEEAKKAYPDAFVRIIGFDNVRQVQLISFIAYKPP
Jpred: ——HHHHHHHHHHHHHH—EEE—EEE—EEE—
Conf: 7776653356765677627899999998625885089987157631688999872588

Query: GC
Jpred: —
Conf: 99

The complete set of outputs can be viewed at:
http://www.compbio.dundee.ac.uk/jpred4/cgi-bin/chklog?jp_yrbsHd9

Jpred batch job T7 completed

 www-jpred@compbio.dundee.ac.uk
to me

Your batch job T7 has completed (with 25 successes out of 25 submitted jobs).

An archive with all the results can be downloaded from the following link:

http://www.compbio.dundee.ac.uk/jpred4/results/jp_batch_1421326154_T7_7jrCepX/jp_batch_1421326154_T7_ALL_JOBS_ARCHIVE.tar.gz

Results for individual queries are available from the links below:

SUCCESS UniRef50_P19084 Link to results: http://www.compbio.dundee.ac.uk/jpred4/results/jp_batch_1421326154_T7_j9E7bxA
 SUCCESS UniRef50_Q43495 Link to results: http://www.compbio.dundee.ac.uk/jpred4/results/jp_batch_1421326154_T7_78UvMeL
 SUCCESS UniRef50_Q9XHP0 Link to results: http://www.compbio.dundee.ac.uk/jpred4/results/jp_batch_1421326154_T7_1MsJ_NG
 SUCCESS UniRef50_P68251 Link to results: http://www.compbio.dundee.ac.uk/jpred4/results/jp_batch_1421326154_T7_yCcKUyk
 SUCCESS UniRef50_P80438 Link to results: http://www.compbio.dundee.ac.uk/jpred4/results/jp_batch_1421326154_T7_h8pBubm
 SUCCESS UniRef50_Q6EUP4 Link to results: http://www.compbio.dundee.ac.uk/jpred4/results/jp_batch_1421326154_T7_J8v3lEI
 SUCCESS UniRef50_P14009 Link to results: http://www.compbio.dundee.ac.uk/jpred4/results/jp_batch_1421326154_T7_v9EqpeL
 SUCCESS UniRef50_P16536 Link to results: http://www.compbio.dundee.ac.uk/jpred4/results/jp_batch_1421326154_T7_5Hc1nTb
 SUCCESS UniRef50_P62258 Link to results: http://www.compbio.dundee.ac.uk/jpred4/results/jp_batch_1421326154_T7 KEANbeO
 SUCCESS UniRef50_Q23878 Link to results: http://www.compbio.dundee.ac.uk/jpred4/results/jp_batch_1421326154_T7_KeU901
 SUCCESS UniRef50_Q2R1D5 Link to results: http://www.compbio.dundee.ac.uk/jpred4/results/jp_batch_1421326154_T7_QGlyQHd
 SUCCESS UniRef50_P22882 Link to results: http://www.compbio.dundee.ac.uk/jpred4/results/jp_batch_1421326154_T7_GSS1QX9
 SUCCESS UniRef50_P13813 Link to results: http://www.compbio.dundee.ac.uk/jpred4/results/jp_batch_1421326154_T7_bdwMsD
 SUCCESS UniRef50_P46730 Link to results: http://www.compbio.dundee.ac.uk/jpred4/results/jp_batch_1421326154_T7_WZrHM2R
 SUCCESS UniRef50_P21215 Link to results: http://www.compbio.dundee.ac.uk/jpred4/results/jp_batch_1421326154_T7_zg5X6rd
 SUCCESS UniRef50_P0A5B8 Link to results: http://www.compbio.dundee.ac.uk/jpred4/results/jp_batch_1421326154_T7_GUURMue
 SUCCESS UniRef50_P18646 Link to results: http://www.compbio.dundee.ac.uk/jpred4/results/jp_batch_1421326154_T7_F9QdnQq
 SUCCESS UniRef50_Q05349 Link to results: http://www.compbio.dundee.ac.uk/jpred4/results/jp_batch_1421326154_T7_CB9jOqi
 SUCCESS UniRef50_P05079 Link to results: http://www.compbio.dundee.ac.uk/jpred4/results/jp_batch_1421326154_T7_n8x16UA
 SUCCESS UniRef50_P16909 Link to results: http://www.compbio.dundee.ac.uk/jpred4/results/jp_batch_1421326154_T7_i9gGzDU
 SUCCESS UniRef50_P81928 Link to results: http://www.compbio.dundee.ac.uk/jpred4/results/jp_batch_1421326154_T7_oLXH90P
 SUCCESS UniRef50_P13744 Link to results: http://www.compbio.dundee.ac.uk/jpred4/results/jp_batch_1421326154_T7_eWuV1w7
 SUCCESS UniRef50_P32234 Link to results: http://www.compbio.dundee.ac.uk/jpred4/results/jp_batch_1421326154_T7_8x6e_I2
 SUCCESS UniRef50_P15455 Link to results: http://www.compbio.dundee.ac.uk/jpred4/results/jp_batch_1421326154_T7_vdcL2N5
 SUCCESS UniRef50_Q8GBW6 Link to results: http://www.compbio.dundee.ac.uk/jpred4/results/jp_batch_1421326154_T7_7jrCepX

Figure 3. (1) Illustration of a single sequence job submission secondary structure prediction results summary email with link to full result details (2). (3) Illustration of a batch submission email summary with overall and per job (4) details that give links to individual predictions and an archive with all results for all sequences submitted in the batch.

v.2014.07 (34). In addition to retraining, the HMM building step in JNet was updated to HMMer 3 (35) and some improvements were made to the code to simplify management and future algorithmic developments. The final accuracy of JNet 2.3.1 was assessed in a blind test on 150 sequences from 150 superfamilies not used in training. The 150 superfamily sequences were selected to reproduce a similar distribution of secondary structure compositions as the training structures in order to avoid biasing the reported ac-

curacy of the blind test results. On the blind test, the average secondary structure prediction Q₃ score increased to 82.0% from 81.5% for JNet v.2.0, and solvent accessibility prediction accuracy rose to 90.0, 83.6 and 78.1% from 88.9, 82.4 and 77.8% for JNet v.2.0 for each of >0, >5 and >25% relative solvent accessibility thresholds.

JPred4 results reporting

JPred3 has been widely used in teaching and integrated into many bioinformatics pipelines across the world. Accordingly, in order to maintain support for legacy courses and scripts, the results options in JPred4 include all the original formats and styles (PDF, HTML, etc.) as well as the intermediary processing files. In addition to these outputs, JPred4 reports have been enhanced to include more visualization options and to present a complete picture of the alignment generated for prediction including all insertions.

Figure 2 summarizes the main results page while Figure 3 shows examples of summary emails returned to a user for single or batch sequence submissions. Unlike previous versions of JPred, the primary visualization of a JPred4 prediction result is a scrollable SVG image. The SVG is generated by Jalview 2.9 (www.jalview.org) (36) run in command-line mode as part of the JPred4 web server processing pipeline so users do not need to run Jalview on their own computers. However, the JalviewLite Java applet result page is still provided for users working with Java-enabled browsers who prefer direct access to Jalview's sophisticated functions.

In all previous versions of JPred, the alignment returned showed the full-length query sequence without gaps necessary to accommodate insertions in sequences returned from the PSI-BLAST search. JPred4 introduces options to view the full multiple alignment including all residues in all sequences or download it for further analysis. For users who have local installations of Jalview (36), Jalview feature files are provided to allow easy annotation and analysis of the alignment and predictions.

In JPred3, a batch job with multiple query sequences would return separate emails for each query. JPred4 condenses these messages into a single email with a summary of success/failure for each sequence (Figure 3) in the batch and a compressed archive of all the predictions.

All JPred4 jobs are currently stored on the server for 5 days.

Time required to complete predictions

The median time for a JPred4 prediction to return results is 5 min calculated over a recent 50 000 consecutive predictions performed by end-users in the autumn of 2014. However, the server can accommodate jobs of up to 3-h duration. Most of the time is spent in the PSI-BLAST search phase which is avoided if the user submits a pre-existing MSA. MSA predictions typically return results within a few seconds.

In summary, the JPred server has been upgraded to provide a richer user experience and to include more accurate secondary structure and solvent accessibility predictions from the JNet 2.3.1 algorithm.

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